1962

Entomogenous fungi of corn insects in Iowa

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ENTOMOGENOUS FUNGI OF CORN INSECTS IN IOWA

by

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A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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Of Science and Technology
Ames, Iowa

1962

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INTRODUCTION

Certain entomogenous fungi (Beauveria, and Metarrhizium) have been employed in recent years in Iowa as microbial insecticides against some of the corn pests occurring here (York 1958, and Smith 1961). There has been little work, however, on fungi which might occur endemically among corn insect populations in Iowa.

It would be of value to the entomologist to be able to identify the fungi he finds growing on the insects with which he is working. This would be especially advantageous when fungi appear in laboratory cultures of insects being mass reared for experimental purposes. Huge population losses often occur due to fungus epizootics within the insect rearing rooms (Boyce and Fawcett 1947). If these organisms were recognized as soon as they began to appear, precautions could be taken to control and then eliminate the disease.

The identity and possible uses of entomogenous fungi, as well as culture techniques, would be of value in future biological control work. Such information would aid in recognizing natural epizootic outbreaks in ecological investigations. Also, with integrated control programs, the information would be utilized in deciding the need for chemical or biological insecticide applications.

Knowledge of the fungi which occur on corn insects in Iowa is of interest from a purely scientific point of view. The addition of host relationship information concerning the fungus species found in the state may aid in an understanding of natural population suppressors.

The purpose of this study was to isolate and identify the fungi and

attempt to clarify the pathogenic nature of the organisms which occur on corn insect pests in Iowa.

REVIEW OF LITERATURE

Insect Diseases

Abnormalities have been observed for centuries in insects such as the honey bee and silkworm. Steinhaus (1956) gave an excellent account of the earliest observations of insect diseases beginning with those of Aristotle (about 335 B.C.).

Diseases of insects caused by fungi were the earliest to be recorded due to their macroscopic size, and rather spectacular effect on their host. In 1835, Bassi de Lodi demonstrated that one disease of the silkworm was caused by a fungus (Steinhaus 1956). During the last half of the 19th century there were many reports of fungi on insects (Steinhaus 1956).

The first use of a fungus as a biological insecticide was by Metchnikoff (1879) in Russia. He reported the destruction of the larvae of a beetle, Anisoplia austriaca Hbst., with the fungus, Metarrhizium anisopliae (Metch.) Sorokin. Metchnikoff's work led to the establishment of the first insect pathology laboratory at the University of Odessa, USSR, in 1884. Krassilstschik established the laboratory for the cultivation and experimental use of entomogenous fungi against insect pests.

During this same period, in the United States, fungi were being studied for use in control measures for insect pests. Hagen (1879) suggested the use of yeast spores for control of insects. Snow (1890) reported on the use of the white fungus, Beauveria globulifera (Speg.), and Empusa aphidis (Hoff.) against the chinch bug, Blissus leucopterus (Say),

in Kansas. Forbes (1895) also carried out experiments with Beauveria globulifera, against the chinch bug in Illinois. Steinhaus (1956) gave an inclusive historical report on the use of disease organisms as control measures against insect pests. Baird (1958) listed 300 articles, with abstracts, on the use of fungi in insect control. Sweetman (1958) gave a very good account of the successful uses of disease organisms against insects. Charles (1941) published a list of over 300 species of fungi that had been recovered from insects in North America.

The genera of fungi discussed in this study have, for the most part, been previously reported from insects in many regions of the world. Some of them, however, are reported as entomogenous in this work for the first time.

Aspergillus

Charles (1941) listed 12 species of Aspergillus as entomogenous on 21 different species of insects. She listed eight species from one insect, the honey bee Apis mellifera Linn. The honey bee has been more intensely studied over the centuries than most insects. Thorough investigation of others will probably bring to light many species of fungi not previously known to occur on those insects.

Speare (1912) described a species of Aspergillus from the sugar cane mealy bug, which he named A. parasiticus. The organism was used in pathogenicity experiments against the mealy bug with inconsistent results. Tests conducted with the same strain of fungus, under nearly identical conditions, produced mortality rates which varied from 5 to 90 percent.

Metalnikov and Toumanoff (1928) carried out pathogenicity experiments against the European corn borer, Ostrinia nubilalis, with the fungus Aspergillus flavus Link. Their results showed a consistent 100 percent mortality among the corn borer larvae. Burnside (1930) in his study of fungi that were parasitic on the honey bee, reported that the bees were parasitized by four species of Aspergillus: A. flavus, A. fumigatus Fres., A. ochraceus Wil., and A. parasiticus. Janisch (1938) recovered Aspergillus versicolor (Vuill.) Tiraboschi from dead larvae of the nun moth in Germany. Boyce and Fawcett (1947) isolated an Aspergillus that was causing an epizootic disease among insectary-reared mealy bugs in California. Laboratory experiments showed 95 to 100 percent mortality among mealy bugs that were used as test animals. The fungus was determined to be in the Aspergillus flavus group, very close to A. parasiticus, but was more brown in color than the green A. parasiticus and had smooth rather than pitted conidia. The organism was not named.

Aspergillus gracilis Bainier (A. depauperatus Petch) was recovered by Rockwood (1951) from dead aphids in Oregon. Steinhaus (1952) listed Aspergillus flavus, and A. niger from larvae of Ostrinia nubilalis, sent to him from Iowa and Ohio. An infection of Platysamia cecropia by A. flavus was discussed by Sussman (1952).

Evlakhova (1953) carried out field experiments against Eurygaster integriceps with Aspergillus repens which resulted in 57 percent mortality among the overwintering bugs. Schmidt (1960) described an infection of the forest ant, Formica polyctena, by an Aspergillus belonging to the A. flavus group. The bedbug, Cimex lectularius Linn., was found by Cockbain

and Hastie (1961) to be parasitized by Aspergillus flavus. Other species of this genus that have been reported as entomogenous (Charles 1941) are: A. depauperatus Petch, A. effusus Tiraboschi, A. flavescens Wreden, A. nidulans (Eidam) Wint., A. oryzae (Ahlburg) Cohn, A. sydowi (Bainier and Sartory) Thom and Church, and A. tamarii Kita.

Beauveria

The genus Beauveria is usually placed with the Fungi Imperfecti, but Schaerffenberg (1955) claimed to have found the ascus form of the fungus and placed it as a new genus in the Ascomycetes. MacLeod (1954) completed an extensive study of this genus and came to the conclusion that there were, at most, only two species in the genus. These two species were B. bassiana and B. tenella. The only distinguishing feature between the two was that the former species had 56 percent globose spores, and the latter had 2 percent globose spores; 98 percent being oval in shape. This is a rather narrow distinction, and probably there is only one species in the genus. In this study, all of the strains of this genus had primarily globular spores, so all were identified as Beauveria bassiana (Bals.) Vuill.

The genus Beauveria was established in 1912 by Vuillemin, in honor of Beauverie, who, in 1911, had pointed out that the characteristics of this entomogenous group warranted its recognition as a new genus. Until that time these fungi had been variously placed in the genus Botrytis, Sporotrichum, or Isaria.

This fungus (Botrytis bassiana) first received attention when it was

identified as the causal agent of the silkworm disease (muscardine de ver soie) in France in the early 19th century. Even now the disease still causes heavy losses in the silkworm industry. In Italy in 1926, over 6,000,000 kilograms of cocoons valued at more than 150,000,000 lira (about $225,000) were lost to this fungus disease (MacLeod 1954).

Even though the fungus has often been a pest, it also has been of great benefit in the natural control of many insect pests. Charles (1941) listed over 140 host insects for this fungus in North America. Baird (1958) reported the use of Beauveria as a biological insecticide against 32 different species of pest insects. Good control was obtained in some cases.

Howard (1902) attempted to control grasshoppers with Beauveria globulifera, and reported success under certain favorable weather conditions. Billings and Glenn (1911) made a study of the control attempts with Beauveria in Kansas against Blissus leucopterus, and came to the conclusion that they were, in general, not successful. They also concluded that if the fungus were present in a given area, the incidence of the fungus could not be increased by the artificial dissemination of the spores. Lefebvre (1931) indicated that a partial control of Ostrinia nubilalis could be obtained by dusting fields with spores of Beauveria bassiana. Experimental work done in Ontario by Stirrett et al. (1937) showed that a maximum of 67 percent control of larvae of Ostrinia nubilalis could be obtained by dusting the corn plants with spores of Beauveria bassiana. The dissemination of spores of B. bassiana on the foliage of apple trees was shown by Janes and Marucci (1947) to significantly reduce the populations of codling moth, Carpocapsa pomonella

(Linn.), larvae on the trees. Dresner (1949) published an extensive piece of work on Beauveria bassiana which included the host range of the fungus, artificial culture methods, moisture requirements, and laboratory and field applications of the spores. Beauveria bassiana was recovered by Steinhaus (1952) from cadavers of European corn borer larvae sent to California from Iowa and Ohio. In Minnesota, Eilingboe et al. (1957) found the sweetclover weevil, Sitona cylindricollis Fahr., infected with B. bassiana. Pathogenicity tests conducted by the group showed that both larvae and adults were capable of becoming infected with the fungus. York (1958) obtained from 42 to 88 percent population reduction among larvae of Ostrinia nubilalis on corn by the application of Beauveria spores to the plants. Smith and York (1960) found that moths as well as the larvae of the European corn borer could be infected with the fungus B. bassiana. Smith (1961) conducted laboratory and field experiments with B. bassiana and found that he could obtain complete control of Ostrinia nubilalis by three granular applications of spores to the corn plants.

Fusarium

Fusarium neoceras Woll. and Reink. has not been previously reported from insects, but many other species of Fusarium have been. Petch (1921) reported his isolation of F. epicoccum McAlp. from the red scale, Aspidiotus aurantii (Mask.). Charles (1941) listed five species of Fusarium as being entomogenous: F. aleyrodis Petch, F. larvarum Fckl., F. merismoides Cda., F. poae (Pk.), and F. solani (Mart.). The microconidial form of

this organism is a Cephalosporium (Buchanan 1911), and some species or strains may not produce the macroconidia which is necessary for inclusion in the genus Fusarium. Petch (1924) reported an entomogenous fungus which he identified as Cephalosporium lecanii Zimm. His description of this organism was an almost exact description of the microconidial stage of Fusarium neoceras. The conidia of Cephalosporium lecanii were described as being formed in chains, which are later pulled into the typical heads by the formation of mucilage around the spores. When this mucilage dries, it leaves a wrinkled dry ball with the spores inside. See Figure 4 (A, B, and D). Baird (1954) reported a species of Cephalosporium from the larvae of Ostrinia nubilalis in Canada. Charles (1941) listed two entomogenous species of Cephalosporium from North America: C. lecanii, and C. longisporum Petch.

Fusarium species have been employed in insect control attempts during the first half of this century. Berger (1910) and Watson (1913) reported Fusarium aleyrodis Petch attacking whiteflies in Florida. Watson attempted artificial dissemination of the fungus spores with good results. Morquer and Nysterakis (1944) employed Fusarium lateritium Nees, in field experiments against Phylloxera vitifoliae (Fitch) in France, with results of 40 percent mortality among the aphid population.

Metarrhizium

This is a very common cosmopolitan genus of entomogenous fungus. It parasitizes a wide variety of hosts among several orders of insects. Charles (1941) listed 64 insect species which were host to Metarrhizium in

North America.

Metarrhizium has frequently been employed, since its discovery by Metchnikoff in 1879, as a biological insecticide against many varied insect pests. Baird (1958) reported Metarrhizium being used in control attempts against 20 different species of insect pests.

Pettit (1895) first reported M. anisopliae (Metsch.) Sorokin in the United States from the wheat wireworm, Agriotes mancus (Say), in New York. Speare (1912) reported M. anisopliae from the sugar cane borer, Rhabdocnemis obscura Boisd., and on Adoretus umbrosus Babr. Hooker (1913) reported control of the larvae of may beetles in Puerto Rico by the artificial dissemination of the spores of M. anisopliae. Wallengren and Johansson (1929) conducted pathogenicity experiments with M. anisopliae against Ostrinia nubilalis, resulting in 100 percent mortality among the treated larvae. In 1930, M. anisopliae was applied against the European corn borer by Hergula. He also obtained good control of this pest. Burnside (1930) during the course of his work on the fungus diseases of the honey bee, Apis mellifera, recovered M. anisopliae from dead bees. He conducted pathogenicity tests with the fungus in order to determine its effects on the bee. The results of these experiments showed that less than 10 percent of the bees became infected with the fungus. Rockwood (1950) recovered Metarrhizium from wireworms in the Pacific Northwest. Fox and Jaques (1959) attempted to control wireworms in Canada with M. anisopliae but were unsuccessful. The only work with Metarrhizium in Iowa on insects was the work by Smith (1961) utilizing M. anisopliae for the control of Ostrinia nubilalis in corn. The results of

these experiments showed that Metarrhizium was very pathogenic to corn borer larvae in the laboratory, but less pathogenic under field conditions.

Petch (1931) published a good taxonomic treatment of this genus, with good descriptions of the known species.

Mycoderma

Mycoderma clayi seems to be a host specific organism, found only on the European corn borer, Ostrinia nubilalis. The only reports of this organism were from this host (Metalnikov et al. 1928, Steinhaus 1952). The latter paper was on micro-organisms from O. nubilalis sent to Steinhaus from Iowa and Ohio.

Paecilomyces

This genus has not previously been reported from insects. It is very similar to the genus Spicaria which does have many representatives in the entomogenous group. Some members of this genus have been described under the genus Spicaria, (Spicaria divaricata), (Gilman and Abbott 1927).

Penicillium

This genus has been reported infrequently from insects. Charles (1941) listed P. cyclopium and one unidentified species of Penicillium as being entomogenous in North America. Frobisher (1926) isolated a species of Penicillium that was an occasional parasite of Drosophila melanogaster. Burnside (1930) reported that Penicillium glaucum was occasionally recovered from dead honey bees. Steinhaus (1949) reported

that six species of Penicillium were entomogenous in North America, but he did not list them.

Rhizopus

Rhizopus nigricans (R. stolonifer) was reported by Charles (1941) from the larvae of a species of Sphingidae. This is the only report of this organism as being entomogenous.

Scopulariopsis

This genus has been reported as being parasitic for man and other vertebrates. It has not been reported from insects. The genus is cosmopolitan; species have been reported from soil, stored grain, leather, exposed fabrics, and cheeses.

MATERIALS AND METHODS

Origin of Fungal Isolates

Various entomologists at Iowa State University of Science and Technology and at the United States Department of Agriculture Corn Borer Laboratory located at Ankeny, Iowa, collected dead and diseased insects as they encountered them in various parts of the state. During the course of this study, over 200 insects were examined and determined to be infected with fungi.

Examination of Cadavers

The dead insects were examined under a dissecting microscope, providing up to 115 diameters magnification, to determine the generic groups to which the fungi belonged. Observations were made on the general growth form and method of sporulation for later comparison with the culture obtained on artificial media. Rough sketches were often made of the conidia and conidiophore apparatus for future reference.

In the latter part of this study, the method worked out by Butler and Mann (1959) for mounting fungi which are plant pathogens, was used on the fungus organisms from insect cadavers with great success. This method involves the use of a short strip of cellophane tape about 2 centimeters wide, which is pressed down over the fungus-covered insect. When the tape is pulled up, many of the conidia and conidiophores adhere to the tape without shattering. A small drop of aniline blue dye is added to the tape on the conidia. After 1 or 2 minutes, the excess dye is blotted away. The

stain greatly increases the clarity of the mount. The tape is then drawn down over a drop of lacto-phenol on a glass slide (adhesive side down). Another drop of lacto-phenol is placed on top of the tape and a cover glass placed over the mount. When examined under a compound microscope, it is possible to see the size, shape and texture of the conidia and also the manner in which the conidia are borne on the conidiophore. This technique often eliminates the necessity for culturing the fungus on artificial media, or on a culture slide. Although Butler and Mann recommended for their photo-micrography work that the tape be placed on the slide adhesive side up, it is easier to handle if the tape is placed adhesive side down. There appears to be no difference in clarity.

Culturing and Identification of Fungal Isolates

The insect cadavers were soaked in a 5 percent solution of sodium hypochlorite for 3 minutes to surface sterilize them. They were then rinsed in sterile distilled water and placed in a petri dish containing Sabouraud's dextrose agar. The dishes were incubated at 27°C. and 60 percent relative humidity to allow the fungus to grow. A few of the organisms failed to grow, and bacterial growth usually overran these within a few days, preventing salvage of the organism for trials on other types of media.

Some of the organisms grew but failed to sporulate. These organisms were transferred to various media in an attempt to induce sporulation. Some of these efforts met with success, but other cultures were eventually lost without ever having sporulated. Difficulty was encountered with Met-

arrhizium anisopliae (Metsch.) Sorokin in particular. Sporulation was finally obtained by growing the organism on a 2 percent agar base, and also on a 2 percent agar base containing 10 grams of Borden's Esbilac per 1000 cc of water. The fungus sporulated well on both of these media. With some strains of Fusarium neoceras only microconidia could be obtained on Sabouraud's dextrose agar. These cultures were transferred to many other types of media in an effort to obtain production of macroconidia. Some of the other media used for fungus culturing in this study were: potato dextrose agar, corn meal agar, Littman oxgall agar, Czapek's agar, nutrient agar, blood agar, brain heart infusion agar, and Thompson's agar. Formulae for the media used in this study are given in the appendix.

Culture slides were made of the organisms which sporulated, using the medium on which they grew best for species determination. Glass slides and 22 x 40 mm No. 1 cover glasses were sealed in small pieces of paper toweling, and sterilized in the autoclave. The slides were then stored in a transfer box until needed. A small cube of culture medium was cut with a flamed scalpel, placed on the slide, and immediately covered with one of the sterile cover glasses. The medium was then inoculated from the side with an inoculating needle. The cube was flattened into a very thin layer by pressure on the cover glass which was then fastened in place with two small strips of cellophone tape. The inoculated slide was next placed in a humidity chamber made from a tight plastic box (4" x 7" x 1½" in size), which had a half-inch layer of absorbent cotton in the bottom. The cotton was saturated with water and covered with a thin sheet of polyethylene. The box was placed in an incubator operating at

27° C., to allow the fungus to grow and sporulate. After 3 or 4 days of growth, the slide was removed from the box and examined under a phase-contrast microscope, using up to 970 diameters magnification. The fungus was then identified with the aid of several different keys (Gilman 1957, Barnett 1960, Thom and Raper 1945, Raper and Thom 1949, and Wollenweber and Reinking 1935). A list of fungus organisms recovered from field collected insects, together with their insect hosts, appears in Table 1.

Pathogenicity Tests

Pathogenicity tests were conducted, with the organisms which sporulated, to determine the possible usefulness of these organisms as control measures. Third through fifth instar larvae of the European corn borer, Ostrinia nubilalis (Hübner), were used as test animals in all of the experiments. This insect was used because of the availability of large numbers of larvae needed for conducting such experiments. Experiments that were conducted early in the investigation were accomplished by the use of a modified Dutky micro-injector (Figure 1); experiments conducted in the latter part of the investigation were patterned after the works of Speare (1912), and Boyce and Fawcett (1947).

The micro-injector was made from a standard micrometer with a cog-wheel added to the shaft handle so that it could be turned an exact distance with each depression of the rotation lever. A standard 1 cc tuberculin syringe was fitted into the apparatus for delivery of the spore suspension. The needle for the spore delivery was made by sealing a 30 gauge dental cartridge needle into a 24 gauge luer-lok hypodermic needle

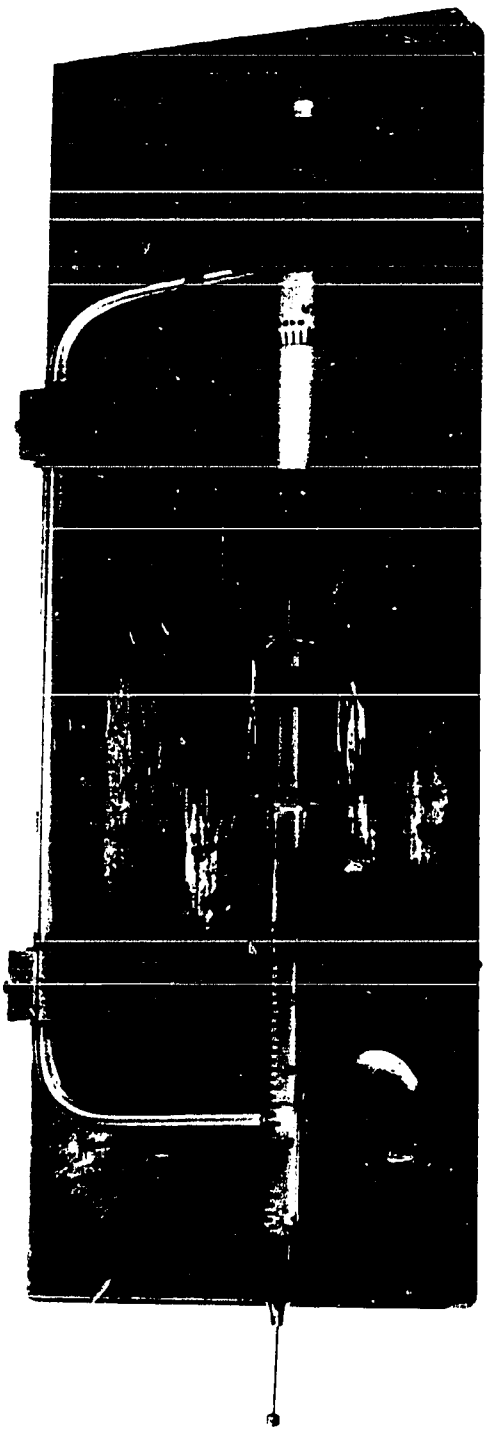
Table 1. Fungi, together with their insect hosts, discussed in this study

Fungus	Insect host	Life form
Aspergillus niger van Tieghem	Ostrinia nubilalis (Hubner)	Larva
Aspergillus parasiticus Speare	Ostrinia nubilalis (Hubner)	Larva Adult
Aspergillus ustus (Bainier) Thom & Church	Ostrinia nubilalis (Hubner)	Larva
Beauveria bassiana (Bals.) Vuill.	Diabrotica longicornis (Say)	Egg Adult
	Diabrotica undecimpunctata howardi Barber	Adult
	Glischrochilus quadrisignatus quadrisignatus	Adult
	Ostrinia nubilalis (Hubner)	Larva Adult
Fusarium neoceras Wollenweber & Reinking	Agrotis ipsilon (Hufnagel)	Larva
	Diabrotica longicornis (Say)	Egg
	Ostrinia nubilalis (Hubner)	Larva
	Phyllophaga sp.	Larva
Metarrhizium anisopliae (Metsch.) Sorokin	Arctiidae	Larva
	Heliothis zea (Boddie)	Larva
	Ostrinia nubilalis (Hubner)	Larva
Mycoderma clayi Met., Ell., & Chor.	Ostrinia nubilalis (Hubner)	Larva
Paicilomyces varioti Bainier	Ostrinia nubilalis (Hubner)	Adult
Penicillium cyclopium Westling	Ostrinia nubilalis (Hubner)	Adult Larva

Table 1. (cont.)

Fungus	Insect host	Life form
Penicillium decumbens Thom	Heliothis zea (Boddie)	Larva Pupa
	Ostrinia nubilalis (Hubner)	Larva
Penicillium puberulum Bainier	Ostrinia nubilalis (Hubner)	Larva Adult
Rhizopus stoloniter (Ehr. ex. Fr.) Vuill.	Ostrinia nubilalis (Hubner)	Larva
Scopulariopsis brevicaulis (Sacc.) Bainier	Heliothis zea (Boddie)	Pupa

Figure 1. Microinjector used in pathogenicity tests

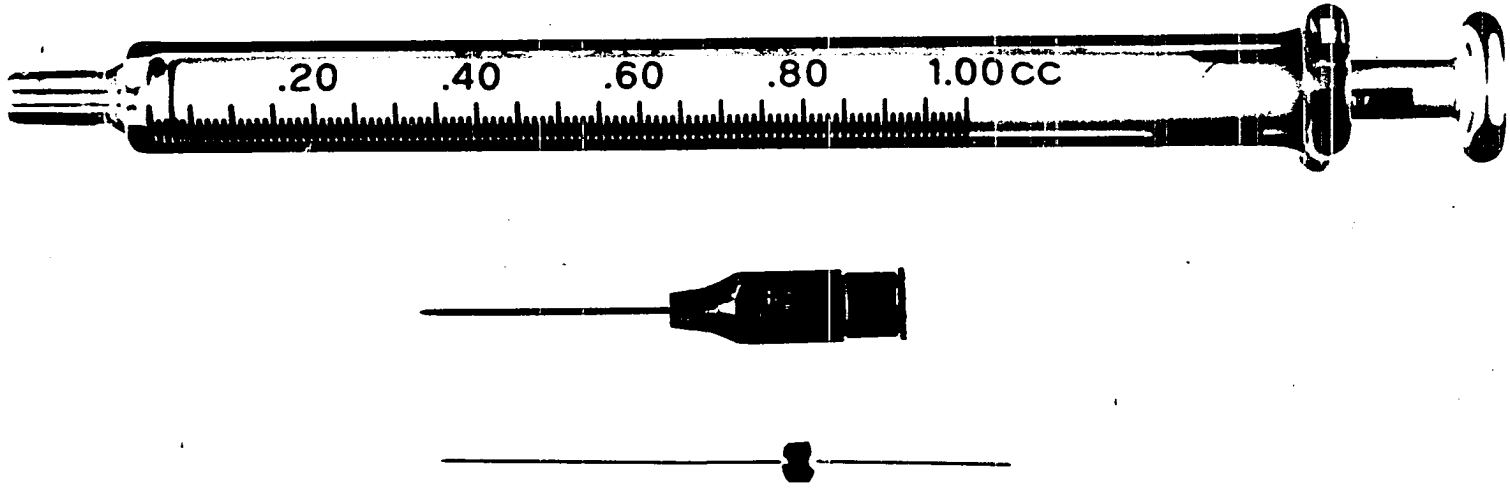


with a small amount of solder (Figure 2). The point of the needle was removed on a fine pumice stone to prevent accidental damage to the insect cuticle.

The application method employing the microinjector was accomplished as follows: A screw-top culture tube containing a slant of Sabouraud's dextrose agar was inoculated with the culture to be tested and allowed to grow for about 2 weeks or until there was a heavy, mature spore-cover on the slant. One cc of sterile distilled water was then added to the tube. Two drops of absolute ethyl alcohol were introduced to the solution in order to break the surface tension and allow a suspension of spores to be formed. When a uniform mixture was obtained, 0.5 cc of the suspension was drawn into the tuberculin syringe which was then positioned in the microinjector.

Each test larva was brought in contact with the tip of the needle, and 0.01 cc of spore suspension extruded by three depressions of the lever. No attempt was made to determine the number of spores delivered, because the number varied between the species of fungi and even between cultures of the same species. These tests were to determine the infectivity of the fungus and not the minimum number of spores required for an infection. After the 10 larvae in each test had been treated, an equal amount of spore suspension was delivered upon the surface of a sterile slant of Sabouraud's agar, to insure that viable spores had been transmitted to the larvae. These inoculated slants were incubated along with the larvae and retained for 10 days at 27° C. and 60 percent relative humidity. Treated larvae were placed individually in shell vials of 2-

Figure 2. Syringe and needles used in the Microinjector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dram capacity. A small piece of green pith from a fresh whorl-stage corn plant was placed in each vial for the larva to feed upon during the observation period. Each vial was stoppered with sterile cotton to prevent cross contamination in the incubator. Ten control larvae, treated in the same manner with sterile water, were incubated with the spore-treated larvae. (Table 2.)

Table 2. Results of infectivity tests applied with the Microinjector

Fungus	Culture No.	Percent mortality	Remarks
Aspergillus niger	560	0	
	576	0	
	622	9	
	647	0	
Aspergillus parasiticus	522	22	
	528	33	
	538	0	
	556	36	
	574	22	
	591	0	
	597	36	
	607	0	
	625	45	
	633	63	
	638	81	
	644	9	
	644	36	
	645	54	
	648	27	
	656	54	
	659	18	
	666	18	
	670	63	
	675	100	
	689	37	

Table 2. (cont.)

Fungus	Culture No.	Percent mortality	Remarks
Aspergillus ustus	594	9	
	618	0	
	691	75	
	698	0	
	713	75	
Beauveria bassiana	310	81	
	755	100	
	760	100	
	760	100	
	775	100	
	Check	100	
	Check	100	
Fusarium neoceras	517	0	Microconidia
	523	22	"
	523	33	"
	528	45	"
	529	12	"
	533	62	"
	751	0	"
	573	0	"
	577	0	"
	578	0	"
	619	0	"
	619	9	"
	621	0	"
	623	0	"
	635	100	"
	636	72	"
	640	33	"
	648	0	"
	648	9	"
	655	14	"
	680	0	"
	717	87	"
	722	100	"
	753	75	"
	759	12	"

Table 2. (cont.)

Fungus	Culture No.	Percent mortality	Remarks
Fusarium neoceras	763	25	Microconidia
Metarrhizium anisopliae	566	55	
	629	75	
Mycoderma clayi	509	0	
	520	9	
	520	0	
	650	0	
Paecilomyces varioti	532	55	
	770	13	
Penicillium cyclopium	587	0	
	587	0	
	614	44	
	624	0	
	624	72	
	762	0	
Penicillium decumbens	610	9	
	654	28	
Penicillium puberulum	768	25	
	771	0	
Rhizopus stolonifer	686	87	

In the latter part of this study the method used by Speare (1912) was followed. Petri dishes of the most favorable medium for the isolate to be studied were inoculated with spores of the test fungus. These were incubated and allowed to grow for a period of about 2 weeks or until a heavy conidial layer covered the colony. The larvae to be used in the test were then placed in the petri dish and allowed to crawl through the fungus for approximately half an hour. The control larvae were placed in

a dish of sterile agar for the same time period. All of the larvae were then removed and placed individually into shell vials containing a piece of corn pith. Theremainder of the test was conducted in the same manner as the previous method. (Table 3.)

Table 3. Pathogenicity tests conducted by allowing test larvae to crawl through a pure culture of the fungus to be tested

Fungus	Culture No.	Percent mortality	Remarks
Aspergillus niger	695	0	
	718	0	
	722	0	
Aspergillus parasiticus	597	37	Spores ex. Insect
	625	45	do.
	645	25	do.
	675	37	do.
	758	11	do.
	765	37	
	765	33	do.
	769	0	
	780	29	
	785	44	
Aspergillus ustus	637	0	
	708	37	
Beauveria bassiana	310	89	
	526	100	
	755	100	
	733	77	
	777	75	
	781	88	
	783	100	
	788	100	
	790	100	
	792	100	
	796	87	
Fusarium neoceras	523	0	Macroconidia
	523	22	Spores ex. Insect

Table 3. (cont.)

Fungus	Culture No.	Percent mortality	Remarks
Fusarium neoceras	621	0	Macroconidia
	623	0	"
	635	33	"
	635	50	Spores ex. Insect
	636	25	Macroconidia
	636	37	Spores ex. Insect
	640	12	Macroconidia
	648	0	"
	655	28	"
	678	0	Microconidia
	696	43	"
	696	12	Macroconidia
	714	12	Microconidia
	717	50	Macroconidia
	722	28	Microconidia
	722	22	Macroconidia
	753	50	Microconidia
	756	0	"
	756	0	Macroconidia
	757	0	Microconidia
	761	0	"
	761	0	Macroconidia
	763	11	"
	764	0	Microconidia
	765	12	"
	776	12	"
	776	33	Macroconidia
	736	11	Microconidia
	791	14	"
	792	12	"
	792	11	Macroconidia
	797	0	Microconidia
Metarrhizium anisopliae	306	55	
	306	66	
	485	71	
	485	100	
	487	88	

Table 3. (cont.)

Fungus	Culture No.	Percent mortality	Remarks
Metarrhizium anisopliae	487	66	
	629	100	
	793	57	
	795	62	
	798	75	
Paecilomyces varioti	532	11	
	770	11	
Penicillium cyclopium	624	0	
	624	0	
	693	0	
	711	0	
	772	0	
	778	22	
Penicillium decumbens	615	0	
	657	0	
	700	0	
	789	29	
	794	37	
	794	0	
Penicillium puberulum	541	12	
	768	25	
	782	25	
Rhizopus stolonifer	686	37	
	752	25	
	752	22	
Scopulariopsis brevicaulis	789	43	
	789	33	

Source of Larvae

The larvae used in the laboratory experiments were reared at the United States Department of Agriculture Corn Borer Laboratory at Ankeny, Iowa, or were overwintering fifth instar larvae, field collected at var-

ious localities within the state. The larvae that were reared in the laboratory at Ankeny were fed on an artificial diet developed by Bottger (1942), and improved upon by Beck et al. (1949) and Becton (1962). This artificial diet contained a mold inhibitor which might have affected the results of the pathogenicity tests. To make certain the mold inhibitor did not affect the test results, the test larvae were removed from the artificial diet and placed on fresh green corn pith for 48 hours prior to treatment. Occasionally a check treatment was run with a known strain of Beauveria bassiana on larvae handled in this manner. One hundred percent mortality was obtained in these check treatments.

The larvae were examined every other day for 10 days and the results recorded. Most of the dead larvae showed evidence of death from a mycosis. The percent mortality among the test insect larvae in these experiments was computed with the aid of Abbott's formula for computing the effectiveness of an insecticide (Abbott 1925).

The photographs of the fungus-covered larvae were made with a 35mm camera, with a bellows-type close-up attachment. The subjects were lighted with four 15 watt fluorescent lamps. Film types were Kodachrome II, and High Speed Ektachrome. The photomicrographs were taken with various film types in a 35mm camera attachment on an American Optical phase-contrast microscope. (See appendix.)

RESULTS AND DISCUSSION

Fungi Collected

Thirteen species of fungi, representing nine genera, were recovered from corn insects during this study. Some of these, such as Beauveria bassiana, Aspergillus parasiticus, and Fusarium neoceras, were recovered frequently. Others, such as Paecilomyces varioti, Scopulariopsis brevicaulis, and Rhizopus stolonifer, were isolated only rarely. The isolates were recovered from eight different species of corn insects. (Table 1.)

The organisms collected in this study may be identified to species in the following key. Only the organisms recovered are included in the key. It, therefore, should be used with caution as new species will be recovered with continued investigation and will not fit into this key. After the organism has been identified using the key, the detailed descriptions should be consulted. Portions of the following key were taken from Gilman (1957).

Key to Species of Fungi

1a. Filaments one-celled, rarely septate; asexual spores typically in a globose sporangium (Figure 6 E and F). No. 12 Rhizopus stolonifer.

1b. Filaments septate; conidia borne on conidiophores, or cells separate and reproduction by budding (yeast-like) 2.

2a. Fungus internal in Ostrinia nubilalis; scattered, yeast-like budding cells (Figure 5 A) No. 7 Mycoderma clayi.

2b. Spores or conidia borne externally on conidiophores 3.

3a. Mature conidia in some shade of green or yellow 4.

3b. Mature conidia in some color other than green or yellow 9.

4a. Tip of conidiophore swollen; conidia often in chains (Figure 3 B) 5.

4b. Tip of conidiophore not swollen; conidia may be in long chains (Figure 4 E and 5 F) 6.

5a. Conidia color yellow, yellow-green, or very light ivy-green; spores globose, rough (Figure 9) No. 2 Aspergillus parasiticus.

5b. Conidia color a very dark green in young cultures, turning brown after a week or 10 days (Figure 8) No. 3 Aspergillus ustus.

6a. Conidia globose to elongate on phialides at tip of conidiophore; heads resembling a paint brush in shape (Figure 5 F), branching symmetrically or asymmetrically 7.

6b. Conidia elongate (Figure 4 F), borne in long chains; pale gray-green in color, (Figure 9); conidiophores in a mycelial mat, very difficult to distinguish individually (Figure 4 E) No. 6 Metarrhizium anisopliae.

7a. Conidia elongate, blue-green in color (Figure 5 E) No. 9 Penicillium cyclopium.

7b. Conidia globose 8.

8a. Penicilli typically in single verticiles of phialides (Figure 5 F) borne on branches which maintain the identity of each verticil. No. 10 Penicillium decumbens.

8b. Penicilli of more than one series of elements branching asymmetrically (Figure 5 D) No. 11 Penicillium puberulum.

9a. Conidia in some shade of brown or black 10.

9b. Conidia hyaline (white) 12.

10a. Conidia black; conidiophore swollen at tip; conidia in chains (Figure 3 F) No. 1 Aspergillus niger.

10b. Conidia in some shade of brown; conidiophore not swollen at tip 11.

11a. Conidia elliptical or slightly elongated, smooth, borne in chains; phialides in loose clusters distributed along the hyphae, sometimes single (Figure 5 C) No. 8 Paecilomyces varioti.

11b. Conidia globose, rough, with a basal constriction or ring, borne in chains from short straight conidiophores (Figure 6 A, B, C, and D) No. 13 Scopulariopsis brevicaulis.

12a. Conidia small, globose, borne in sporodochia, on zig-zag shaped conidiophores; colony very dense and velvety white on the insect (Figure 3 D) No. 4 Beauveria bassiana.

12b. Microconidia short cylindric with rounded ends, one or two celled, borne in chains or in closely packed heads; macroconidia, when present, elongate, slightly curved, with pointed ends, up to nine cells, typically five or six (Figure 4 A, B, and C) No. 5 Fusarium neoceras.

Genus Aspergillus

The genus Aspergillus was erected by Micheli, in 1729 (Thom and Raper 1945). It contains a large number of species. The following descriptions were taken largely from Thom and Raper (1945).

No. 1 Aspergillus niger van Tieghem

Colonies rapidly growing with abundant submerged mycelium, colorless, or in some strains with more or less yellow color in the hyphae and in the substratum, with aerial hyphae usually scantily produced, but abundant in age in certain strains. Conidial heads fuscous, blackish-brown, purple-brown, in every shade to carbonaceous black (Figure 10) varying in intensity with the quantity of pigment produced; typically globose or radiate, commonly up to 300, 500, or occasionally 1000μ in diameter with periphery variously splitting into radiating columns of conidia; small heads, more or less columnar and consisting of a few conidial chains often borne on trailing hyphae or short conidiophores near the substratum. Conidiophores mostly rising directly from the substratum, uncolored or yellow to brown near the vesicle only, smooth, with walls thick, fre-

quently uneven on the inner surface and splitting lengthwise into strips when broken, unseptate or with occasional thin septa, varying greatly in length and diameter in different strains and in colonies on different media or even in sections of the same colony, thus ranging in strains with conidiophores 200 to 400μ by 7 to 10μ to forms with conidiophores several millimeters long and 20μ or more in diameter. Vesicles globose or subglobose, thick-walled, commonly 20 to 50μ, occasionally up to 100μ in diameter, colorless or more commonly more or less intensely yellow-brown. Sterigmata in one series in young colonies and in small heads, but typically in two series, colorless at times, usually more or less intensely brown, even carbonaceous, primary sterigmata closely packed, covering the vesicle, varying greatly in size in the same colony but usually 20 to 30μ in length by 6 to 8μ in diameter at the outer end; secondary sterigmata more uniform, ranging usually from 6 to 10μ by 2 to 3μ, both series often more or less brown to almost black. Conidia globose when ripe, with walls at first smooth with diffused brown or fuscous color, then rough or spinulose from coloring substance deposited as tubercles, bars or loops between the outer primary wall and the inner, or secondary wall, mostly 2.5 to 4μ, occasionally up to 5μ in diameter.

Sclerotia globose, superficial, regularly produced by certain strains, sporadically by some, and not found in many others.

The strains isolated during this study did not produce sclerotia at all. All of the strains produced conidia that were carbonaceous black in color.

A total of seven cultures were isolated in this study, all from the

European corn borer, Ostrinia nubilalis. This is a most distinctive organism on insects, and one of the most easily recognized of the entomogenous fungus species in Iowa (Figure 9). The large black spore heads are easily distinguished with the unaided eye and are unmistakable once observed.

Pathogenicity tests conducted with this organism (Tables 2 and 3) gave mortality rates of from 0 to 9 percent, with an average of less than 2 percent mortality for the seven strains tested. This organism is no doubt a saprophyte, which grows on the carcasses of insects which died of some cause other than the fungus. It is not uncommon on dead larvae of the European corn borer, as it was recovered on 4 percent of the larvae of O. nubilalis examined in this study. The fungus grew well on almost any medium on which it was inoculated, including Sabouraud's dextrose agar.

No. 2 Aspergillus parasiticus Speare

Colonies on Czapek's solution agar with sucrose spreading rapidly, forming a surface growth of crowded conidiophores with very few sterile hyphae, in deeper yellow-green shades near ivy-green; reverse uncolored or yellowish. Conidial heads radiate, abundantly produced and giving color to the colony. Conidiophores given by Speare as 300 to 700μ long, commonly under 400μ, with walls colorless, prominently rougher pitted, enlarging from 3μ at the foot up to 10 to 12μ, and passing into vesicles up to 35μ in diameter. Sterigmata in one series, 7 to 9μ by 2.5 to 3μ, closely packed over the vesicular surface, yellow. Conidia pyriform to globose, very rough, 4 to 5μ, occasionally 6μ in long axis, green (Figure 9). No sclerotia or perithecia reported.

This organism is in the Aspergillus flavus complex. Although several characteristics are given for distinguishing the two species, the morphology seems to overlap, and probably the two are variations of a single species. The species A. parasiticus is reported to be found on insects, has shorter conidiophores than A. flavus, and is a slightly darker shade of green. The strains of A. parasiticus recovered in this study had conidiophores that varied from short to long and color from pale yellow to ivy green; the remaining distinctive feature was that they were found growing on expired insects. A. flavus has been reported from insects, but since A. parasiticus is presumed to be the entomogenous member of this group, and since some of the strains isolated in this study were shown to be parasitic on insects, the name A. parasiticus is used for these fungi in this study.

The group of organisms isolated and identified as Aspergillus parasiticus were typical of the species except for the wide color range of the conidia. They ranged in color from yellow to pale yellow-green to a light ivy-green. A few could be called tan or light brown in color in the older colonies (6 weeks old). Most of the organisms, however, were a pale yellow-green and very typical of the type species described by Speare.

Twenty-five different strains of this organism were isolated from cadavers of the European corn borer, Ostrinia nubilalis. Thirty-one pathogenicity experiments were conducted with them (Tables 2 and 3). Mortality ranged from 0 to 100 percent, with an average rate of 37 percent mortality for the 31 tests.

Inconsistent results were obtained with A. parasiticus in the path-

ogenicity experiments. Results ranged from 37 to 100 percent mortality with the same isolate. Speare had similar results in his experiments with this organism, obtaining from 5 to 95 percent mortality with the same strain of fungus. This variability will probably prevent its being employed as a microbial insecticide. However, since it was recovered from 14 percent of the European corn borers examined in this study, it undoubtedly plays a significant role in the natural mortality of wild populations of O. nubilalis in Iowa. During the course of this study, this organism was found repeatedly on European corn borer larvae which were being reared on artificial diet. The mold inhibitors in the medium appeared not to suppress the growth of this fungus. A. parasiticus, therefore, could prove to be a problem in mass rearing the European corn borer.

No. 3 Aspergillus ustus (Bainier) Thom and Church

Colonies upon Czapek's solution agar spreading broadly, plane, sulcate, or umbonate, rarely zonate, more or less felted or floccose; at first white, becoming olive-gray, yellow-brown, fuscous or russet to purplish vinaceous with the development of mature conidial structures; generally heavy sporing, with some conidiophores arising from the substratum but more abundantly from aerial hyphae; reverse in shades of yellow, orange, and brown to almost black in age; odor not pronounced. Heads radiate to irregularly hemispherical, sometimes loosely columnar (Figure 3 E), commonly splitting into more or less well-defined columns in age, variable in size, ranging in color from dull green or olive-gray, through grayish-brown to fuscous or fuligineus. Conidiophores arising from sub-

merged hyphae ranging up to 500μ long by 3 to 6μ, aerially borne conidiophores, ranging from very short up to 125μ by 2 to 5μ, sinuous, sparsely septate, with walls rather thin, smooth, and uniformly colored some shade of brown. Vesicles hemispherical to subglobose, 8 to 20μ in diameter, smaller in some strains. Sterigmata colorless or colored, semi-radiate, loosely arranged into two series, primary sterigmata 4 to 7μ by 3μ, secondary sterigmata 5 to 7μ by 2.0 to 2.5μ. Conidia globose, 3.5 to 5.0μ, roughened, echinulate to marked with conspicuous color bars, ranging from greenish through olive-gray to yellow-brown or fuligineus. Many strains producing thick-walled hulle cells ranging in form from irregularly ovate or elongate in some strains, to serpentine, helicoid, or twisted in others, essentially as in Aspergillus flavipes.

Seven isolates were made of this organism, all from larvae of the European corn borer, Ostrinia nubilalis. Pathogenicity tests conducted with the organism (Tables 2 and 3) showed a range in mortality from 0 to 75 percent, with an average of 28 percent for the seven strains tested. This organism remained green on the insect and did not turn brown with age as it did on culture media. In gross appearance on the insect it greatly resembled the various species of Penicillium, but it could be distinguished with the aid of a dissecting microscope by the vesicle at the tip of the conidiophore which bears the conidia.

Aspergillus ustus was recovered from slightly less than 4 percent of the European corn borer cadavers that were examined in this study. Since it is not widespread in nature, and since it is of low virulence it probably has little adverse effect on the insect population.

Genus Beauveria

The genus Beauveria was established in 1912 by Vuillemin in honor of Beauverie, who, in 1911, had pointed out that characteristics of this group warranted its recognition as a distinct genus.

According to MacLeod (1954), this genus contained only two species. The two species were B. bassiana, and B. tenella, which were separated according to spore shape. B. bassiana had globose spores and B. tenella had oval-shaped spores. All of the strains isolated in this study had globose spores and were thus identified as B. bassiana. The following description was taken from MacLeod (1954).

No. 4 Beauveria bassiana (Bals.) Vuill.

Hyphae slender, 1.5 to 2μ in diameter, hyaline, septate; colonies flat, mealy, or finely pulverulent with a chalky appearance somewhat like the surface of a newly broken piece of chalk, white to pale cream on the surface, does not color the undersurface of potato dextrose agar; phialides, variable in shape, ventricose to filamentous, develop on the main hyphal branches or on short branchlets at right angles to the main axis, branching may be repeated, forming compact globose heads; spores borne on slender zig-zag conidiophore (Figure 3 D) that extends from an inflated base, globose, 2.4μ in diameter.

This species was isolated from four species of insects in this study (Table 1). Thirteen isolates of the organism were obtained. This organism had many different growth forms. Usually the growth was a thick velvety white mass adhering closely to the insect (Figures 7, 11, and 12);

Figure 3. Photomicrographs of entomogenous fungi

A. Aspergillus parasiticus, conidiophores; approx. 300X

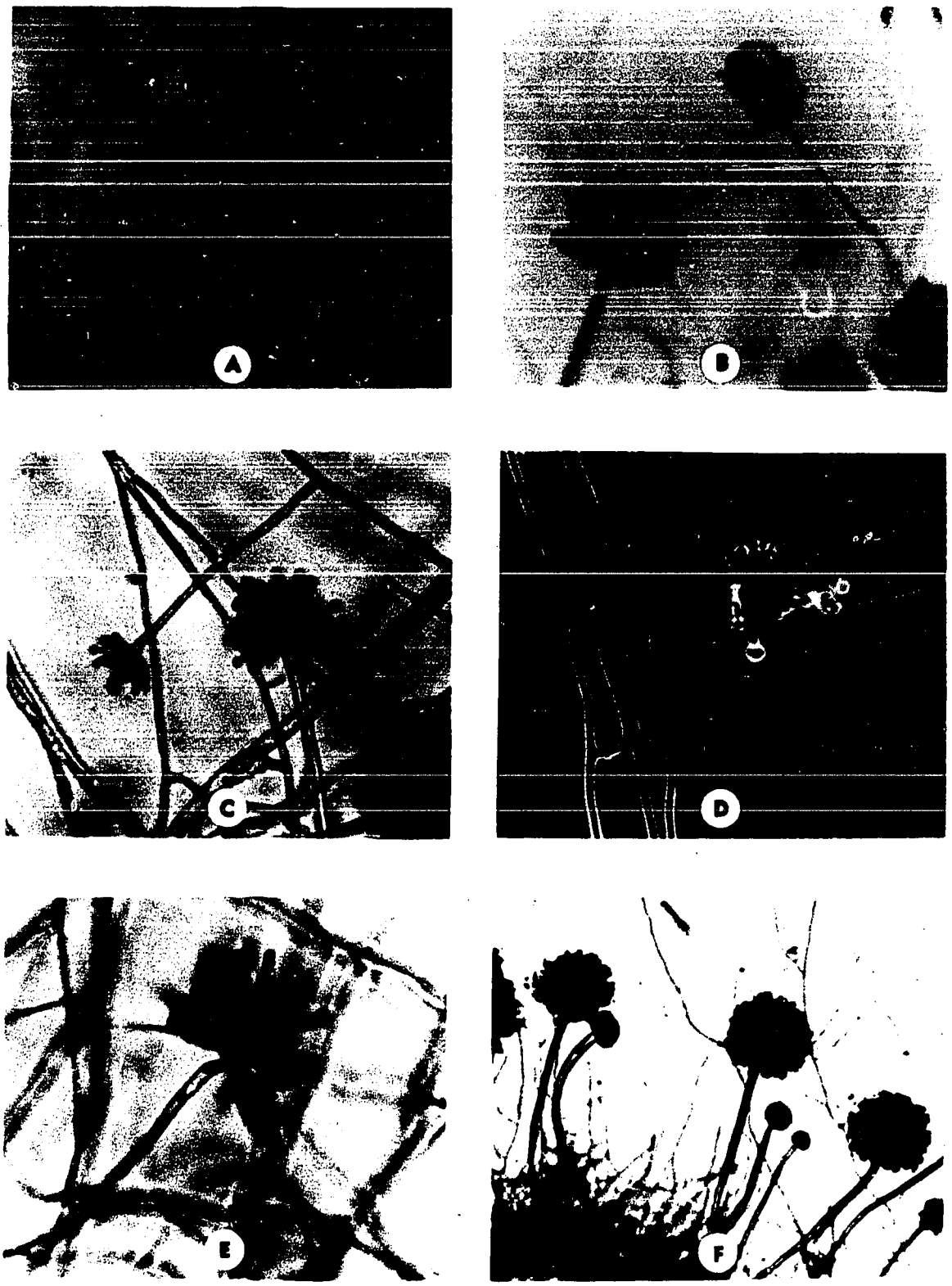
B. Aspergillus parasiticus, conidia; approx. 600X

C. Aspergillus ustus, conidiophores and conidia; approx. 1300X

D. Beauveria bassiana, conidiophores and conidia; approx. 3000X

E. Aspergillus ustus, mature spore head; approx. 1300X

F. Aspergillus niger, mature spore heads; approx. 300X


A
B
C
D
E
F

however, in one case the fungus formed long ropes of hyphae that extended over a centimeter from the insect and greatly resembled the conidial stage of the genus Cordyceps. The thick velvety white growth on the dead insect was usually easy to recognize. The characteristic appearance of Beauveria allows it to be easily distinguished from other fungus species found on insects.

Pathogenicity tests were conducted with the 13 strains of Beauveria isolated from the various insects, using larvae of the European corn borer as test animals (Tables 2 and 3). Mortality rates of 75 to 100 percent were obtained, with an average mortality rate of 94 percent in 18 conducted tests. This organism was recovered from approximately 7 percent of the dead insects examined, and the pathogenicity tests that were conducted showed the organism to be very pathogenic for the insect against which it was employed. The virulence of this organism indicated that it probably is an important natural control factor even though it is not as common in nature as some of the less virulent species of fungi. It was by far the most highly infective organism tested against the European corn borer in this study, and the only one that produced consistently high mortality.

Genus Fusarium

This genus is frequently reported from insects, and was found to be quite common on corn insects in Iowa. Only one species, however, was recovered in this study. The following description was taken from Wollenweber and Reinking (1935), and Gilman (1957).

No. 5 Fusarium neoceras Wollenweber and Reinking

Microconidia single or in false heads, not in chains, one-celled, oval-spindle-shaped, seldom two-celled, exceptionally three-celled, later scattered as dust in mycelium. Macroconidia (Figure 4 C) in sporodochia and pionnotes, brownish-white-cream to incarnate, at times becoming flecked with violet or blue tones, and varying in concentric zones of the stroma, and laid on it in rings, straight or weakly curved, tapering at both ends, slightly constricted at tip, with tenpin so slightly pedicellate base, three-(three-to-five-) very seldom six- to nine septate. Macroconidia measurements are listed below:

0-septate, 5 to 18 x 2.75 to 4.5μ

1-septate, 14 to 34 x 3.25 to 5.5μ

3-septate, 32 to 59 x 3.5 to 5μ

5-septate, 55 to 67 x 4.5 to 5.5μ

6- to 9-septate, 17 to 120 x 4 to 5μ

Chlamydospores and sclerotia lacking

This fungus has not previously been reported from insects. It was first reported from soil and dead banana leaf sheaths in Panama. The above description fits the organism, with a few minor exceptions which are given here. Also, some additional details are added which were not included in the original description.

F. neoceras is closely allied to another species F. moniliforme which is distinguished by the difference in size of the macroconidia, the production of a pink, instead of blue or purple, color in the substratum, and microconidia formed in chains rather than singly and in false heads. These

two organisms are very closely related and are probably variations of a single species, since all types of gradations were found in this group of organisms from corn insects in Iowa. Because most of the forms recovered were best described by the description of F. neoceras, they were placed in this species. The microconidia in this group of organisms are at first formed in chains (Figure 4 A). Later these are pulled into heads by the production of mucilage around the microconidia (Figure 4 B). It is this stage, resembling the genus Cephalosporium, that is most frequently encountered on the insect, and the fungus must be transferred to artificial media before the macroconidia typical of Fusarium are produced.

On culture media various strains of this organism stained the substrate. The color ranged from pure white through yellow to brown, into blue and purple, and from yellow into pale orange and pale to dark pink. On the insect, the color was almost always white; it was on occasion a pale pink or a light orange in color. The growth on media was usually loose and floccose, but occasionally became quite thick and smooth and closely resembled a culture of B. bassiana in gross morphology. This type of growth occurs infrequently on the insect and could be confused with Beauveria, but examination under a dissecting microscope will reveal the absence of sporodochia which are so characteristic of the genus Beauveria. This superficial resemblance to Beauveria in gross morphology has perhaps caused confusion, because this organism is very abundant on corn insects in Iowa. Any white fungus found on insects is likely to be called Beauveria, because this is the only well-known white fungus which occurs on insects. Since the microconidia are most frequently encountered on insects

in this species, and since the microconidia are more easily produced on artificial media, some measurements of the spores were taken and are listed here: microconidia range in length from 4.5μ to 24.5μ and in width from 1.1μ to 7.5μ. The microconidia are usually single-celled, but spores with two cells are frequently seen. Three-celled microconidia are not uncommon. These multiple-celled microconidia are easily distinguished from the multi-celled macroconidia by the much thicker diameter in relation to their total length.

This was the most common organism recovered from insects during the course of this study. Thirty-six isolates were obtained from four species of insects (Table 1). The majority of these organisms were isolated from the European corn borer. Pathogenicity tests conducted with this organism (Tables 2 and 3) showed that in general the organism was not highly pathogenic, although some strains produced up to 100 percent mortality in some tests. A total of 60 pathogenicity experiments were carried out with the 36 isolates and produced an average of 21 percent mortality among larvae of the European corn borer. The organism would be considered mildly pathogenic to saprophytic and probably is an opportunist when the insect has been weakened by some factor in its environment. The high percentage of insects collected that were infected with this organism indicates that the spores of this species must be extremely abundant in nature.

Genus Metarrhizium

This is a small genus with only three or four species. The most well known and cosmopolitan species in the genus is Metarrhizium anisop-

Figure 4. Photomicrographs of entomogenous fungi

A. Fusarium neoceras, chains of microconidia; approx. 1300X

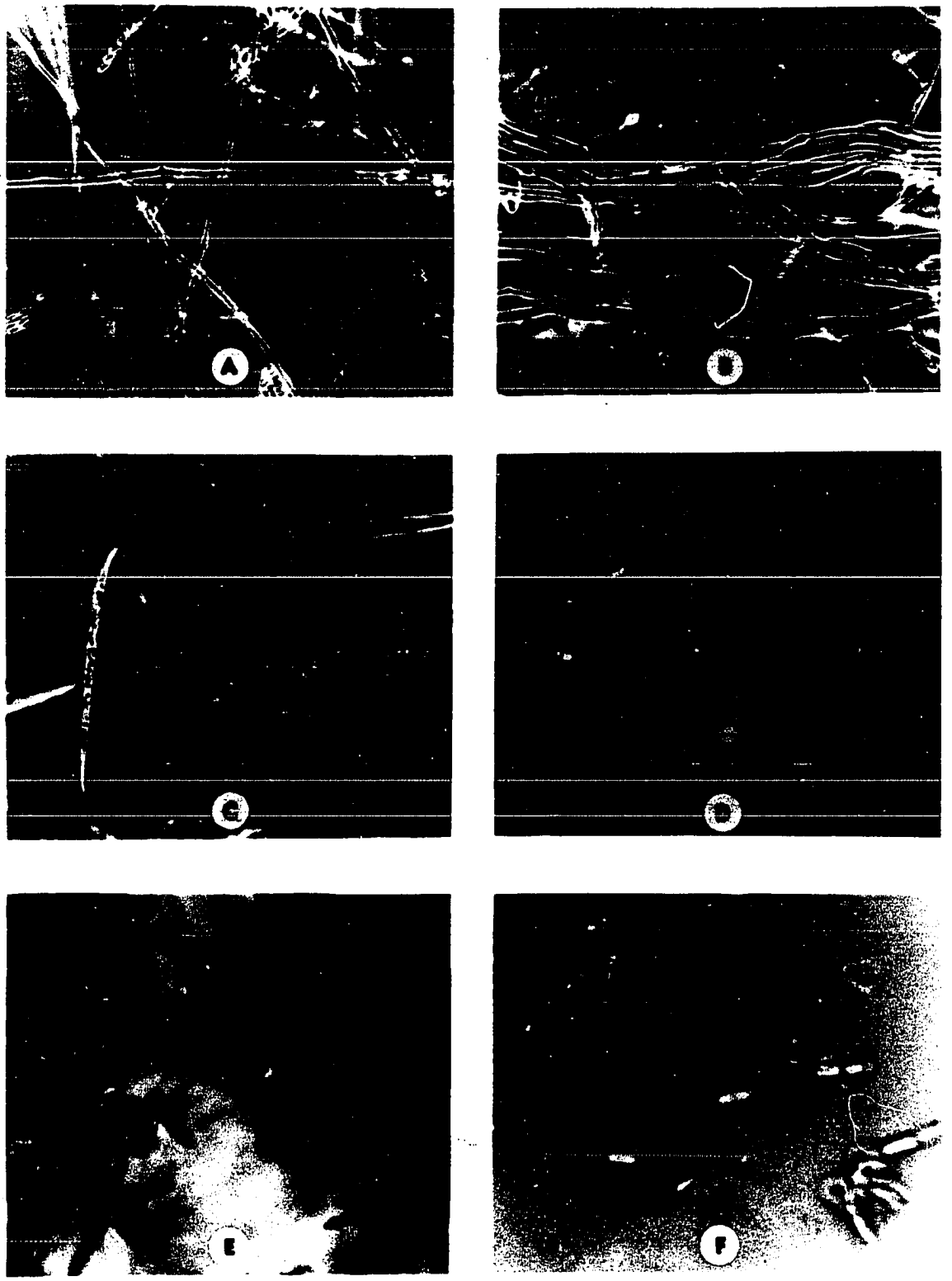
B. Fusarium neoceras, heads of microconidia; approx. 1300X

C. Fusarium neoceras, macroconidia; approx. 3000X

D. Fusarium neoceras, dried mucilage around the microconidia; approx. 1300X

E. Metarrhizium anisopliae, culture on agar; approx. 1300X

F. Metarrhizium anisopliae, conidia; approx. 3000X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liae, which is the only species of the genus recovered during this study. The description which follows was taken from Petch (1931), and Vuillemin (1904).

No. 6 Metarrhizium anisopliae (Metsch.) Sorokin

The fungus forms a large thallus of which the filaments are compressed and branched, forming small hummocks, often confluent and disappearing under the mass of conidia they produced. Conidiophore and the branches, either isolated, in pairs, or in verticils, arise below the septa of the upper part of this stalk. The conidia (Figure 4 E) are formed in basipetal succession, and are united by a disjunctor which is a modification of the membrane, flattened and compressed by the pressure of the growth of new conidia. The resulting conidial chains often reach a length of 800μ or more. The conidia are olive green in color (Figure 9), cylindrical, 4 to 15μ by 2 to 3.5μ, with rounded ends (Figure 4 F). Chlamydospores can be obtained on certain artificial media.

Eight strains of this organism were isolated from three different insect species (Table 1). Several other insect cadavers were diagnosed as Metarrhizium kills early in this investigation; however, those determinations could not be checked by propagating the organism on artificial media. Most authors reported luxurious growth on potato slices and potato agar. Only vegetative growth was obtained on these media during these investigations, and spores were never produced. Several insect cadavers, tentatively identified as Metarrhizium kills, were lost in the process of finding a suitable culture medium. This fungus was finally successfully cultivated on a 2 percent agar medium with no added nutrients,

and also the same medium with 10 grams of Borden's Esbilac per 1000 cc of water.

Pathogenicity tests conducted with this fungus on larvae of the European corn borer gave mortality rates of 55 to 100 percent, with an average of 72 percent for 12 tests (Tables 2 and 3).

This organism can be confused only with Aspergillus parasiticus. These two species are the only entomogenous fungi with light green spores. M. anisopliae has pale gray-green spores, and A. parasiticus has pale yellow-green spores. Figure 9 gives a comparative gross morphology of the two species. The other green-spored fungi found on corn insects have a dark blue-green color. Because several isolates of what probably was M. anisopliae were lost in the process of working out culture techniques, it is difficult to make a definite statement concerning the rate of occurrence of M. anisopliae among corn insects in Iowa. Eight isolates were definitely identified as M. anisopliae. Since the pathogenicity rate was high for the group, it can be said that this species probably does contribute an effective amount of suppression upon wild insect populations.

Genus Mycoderma

M. clayi is the only species of this genus known to occur on corn insects. This species is host specific in the European corn borer. The description of the organism was taken from Metalnikov et al. (1928).

No. 7 Mycoderma clayi Metalnikov et al.

A Gram-positive elongated cell, extraordinarily large, measuring 9 to 16μ in length, and 1.5 to 3μ in width. No other microorganism, isolated

from corn borer larvae, has ever approached this size. The morphology of the organism indicated that it is a typical yeast. By staining the living cells, two to four vacuoles with metachromatic granules become visible. The propagation takes place by typical budding. The buds are usually formed at one of the ends of the rod (Figure 5 A). The yeast grows well on most media, such as Sabouraud's medium, Mayer's fluid, and others. Malted water is the most favorable medium.

Usually the yeast degenerates on artificial culture-media, loses its original form and develops a mycelium. In Mayer's fluid it preserves its budding form best. Development of spores has not been observed.

This organism was recovered from three different specimens of O. nubilalis. Pathogenicity tests conducted with M. clayi (Tables 2 and 3) gave no kill of larvae when it was applied to the epidermis of the test insect. One strain of the organism was force-fed to European corn borer larvae, and also failed to kill the insects. It was concluded that M. clayi would be of little value as a microbial insecticide.

Genus Paecilomyces

The genus Paecilomyces was established in 1907 by Bainier for a saprophytic mold which produced verticillately branched conidial structures that superficially resembled those produced by Penicillium, but which lacked green color. The following description was taken from Raper and Thom (1949).

No. 8 Paecilomyces varioti Bainier

Colonies spreading broadly upon all common media, in shades of yellowish-brown, never green, with superficial growth consisting mostly of trailing fertile hyphae or ropes of hyphae, becoming powdery in appearance when mature; reverse of colony not discolored in some strains, developing bluish-green shades in others; fertile hyphae septate, usually short, mostly creeping; conidial fructifications either terminal or on short branches of creeping or partially erect hyphae, consisting of separate sterigmatic cells, or of verticils, or series of verticils of branchlets and sterigmata irregularly distributed along the fertile hyphae; sterigmata 15 to 20μ by 3μ with long acuminate tubes usually bent away from the axis of the cell and widely divergent at the apices, bearing long chains of conidia; conidia elliptical or fusiform (Figure 5 C), 5 to 7μ by 2.5 to 3.0μ, yellowish to brownish, smooth-walled, swelling in germination to 10u and producing two or more tubes. (In culture the organism closely resembles the genus Scopulariopsis, which is included in this study, but the conidia of the two genera are very distinctive.)

Both isolates of this organism obtained in this study were recovered from adults of the European corn borer. None of the 170 larvae examined in this study were found infected with this fungus.

Pathogenicity tests produced from 11 to 55 percent mortality among the larvae of the European corn borer, with an average of 22 percent for four tests conducted (Tables 2 and 3).

Genus Penicillium

Penicillium was established by Link in 1809 (Raper and Thom 1949). Raper and Thom's (1949) monographic treatment of the genus listed almost 150 species. The following descriptions were based on their species discussions.

No. 9 Penicillium cyclopium Westling

Colonies upon Czapek's solution agar usually growing rapidly, attaining a diameter of 4.5 to 5.0 cm. in 12 to 14 days at room temperature; usually more or less radially furrowed, from 500μ to 1000μ deep, azonate or broadly zonate in age, in some cultures tending to develop limited sterile overgrowths, with margin compact, white, 1 to 2 mm. wide during the growing period, often thinning in age, heavily sporing throughout and shading quickly through light bluish or green shades in young conidial areas to deeper shades near bluish gray-green (Figure 13), artemisia green or lilly green at maturity, with surface typically appearing granular or "mealy"; conidiophores arising from the substratum, often crowded into fascicles or tufts, but usually borne more or less separately; exudate lacking in some strains, abundantly produced in others, clear or very faintly colored in pink or orange shades; odor pronounced, "moldy" but difficult to characterize; reverse uncolored or yellowish at first, becoming orange-brown or even purplish in 2 weeks in most strains, remaining essentially colorless in others; penicilli large, about 50 to 60μ in length, asymmetrically branched, bearing tangled chains of conidia in irregular masses up to 150μ long; conidiophores arising from the sub-

stratum, mostly 200 to 400μ in length by 3.0 to 3.5μ in diameter, sometimes coarser, with walls typically roughened but in some strains appearing smooth or nearly so; penicillus usually showing one or occasionally more branches, 15 to 30μ by 2.5 to 3.5μ, often appressed and like the main axis usually bearing 3 to 4 metulae 10 to 15μ by 2.5 to 3.3μ, each supporting a verticil of 4 to 8 sterigmata measuring 7 to 10μ by 2.2 to 2.8μ, with apices ending abruptly in conidial chains; conidia mostly subglobose 3.5 to 4.0μ in diameter but with both globose and elliptical conidia observed, the latter commonly ranging from 3.3 to 4.0μ, by 2.5 to 3.0μ, with walls smooth or delicately roughened.

The forms of this species recovered in this study were typical of the species and the genus except that no globose spores were observed. This species has previously been reported from insects by Charles (1941) but has not previously been reported from the European corn borer, Ostrinia nubilalis. This species can be distinguished from other members of the genus recovered in this study by its darker blue-green color (Figure 13), and by the oval shaped spores (Figure 5 E).

Eight isolates were made of this organism, all from the European corn borer, Ostrinia nubilalis. Pathogenicity experiments conducted with this fungus species produced mortalities of 0 to 44 percent, with an average of 6 percent for 11 tests (Tables 2 and 3). This species is no doubt saprophytic on dead insects and probably contributes little to the suppression of insect populations.

No. 10 Penicillium decumbens Thom

Colonies on Czapek's solution agar slowly spreading, attaining a diameter of 2.0 to 3.0 cm. in 12 to 14 days at room temperature, almost velvety in some strains, in others showing a tendency to develop white mycelial overgrowths in central areas, in still others almost floccose and fairly deep up to 1 to 2 mm. but all characterized by loosely interwoven and trailing hyphae bearing short conidiophores, sporulating over the whole colony surface, marginal growth in some strains very thin, largely submerged in zones from 1 to 3 mm. wide, colored in grayish yellow-green shades near tea green (Figure 13); in older colonies developing surface tufts of sterile secondary mycelium; exudate lacking or inconspicuous; odor distinctive, fragrant, suggesting soap perfumes; reverse colorless or with a slight greenish cast; conidiophores 50 to 100μ by 2.0 to 2.5μ, with apices slightly enlarged, smooth or finely roughened, borne at successive nodes upon trailing hyphae which in marginal areas of many strains grow stolon-like along the substratum; penicilli almost entirely monoverticillate and only occasionally showing a branch, producing loose columns of conidia up to 100μ in length; sterigmata mostly in compact clusters up to 12 or 15 in number, 7 to 9μ by 2.0 to 2.5μ, sometimes borne at two immediately adjacent levels; conidia elliptical to subglobose 2.0 to 2.5μ in long axis, occasionally up to 3.0μ, smooth, appearing slightly green under the microscope.

This organism has not previously been reported from insects. It is saprophytic to weakly parasitic. The pathogenicity tests conducted with this fungus produced mortality rates from 0 to 37 percent, with an average

Figure 5. Photomicrographs of entomogenous fungi

A. Mycoderma clayi, budding cells; approx. 3000X

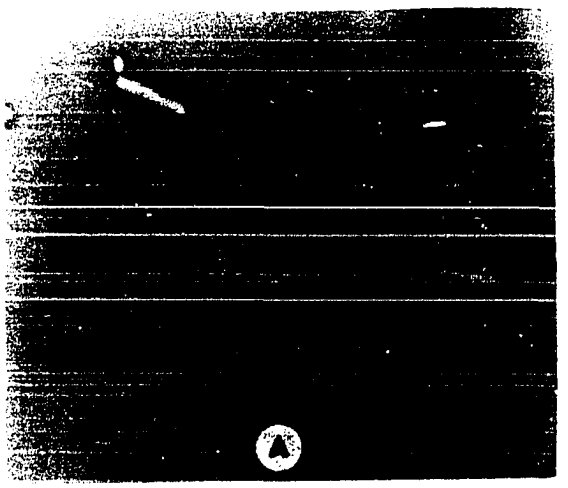
B. Paecilomyces varioti, conidiophores; approx. 600X

C. Paecilomyces varioti, sterigmata and conidia; approx. 3000X

D. Penicillium puberulum, typical penicilli; approx. 1300X

E. Penicillium cyclopium, sterigmata and conidia; approx. 3000X

F. Penicillium decumbens, typical penicilli; approx. 1300X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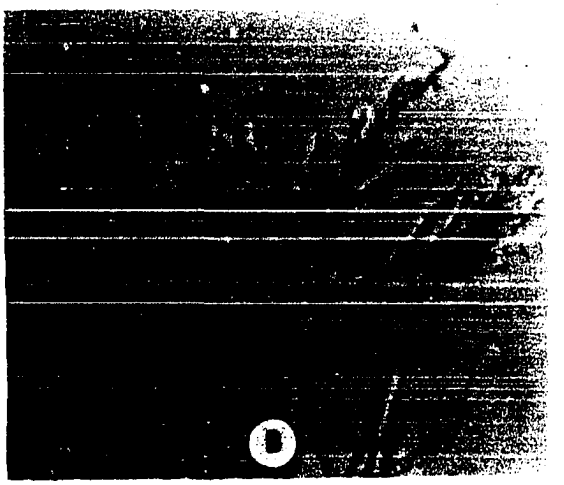
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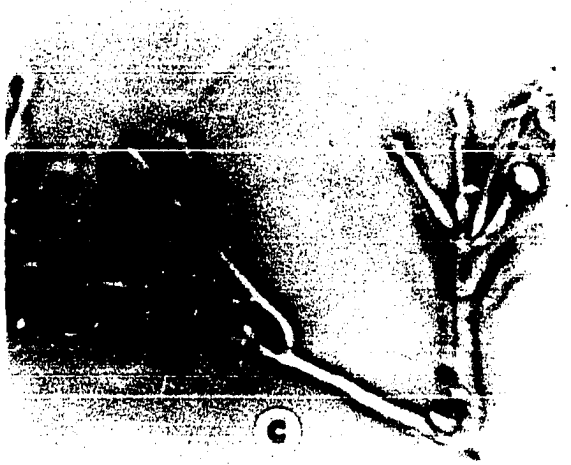
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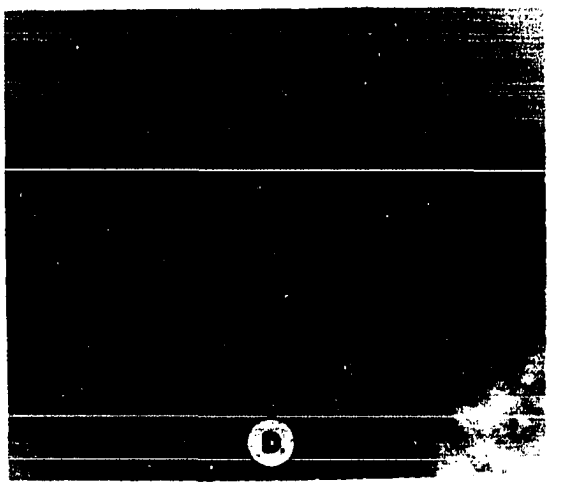
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F

of 13 percent for a total of eight tests conducted (Tables 2 and 3). P. decumbens is distinguished from other members of the genus Penicillium found on corn insects in Iowa by the monoverticillate branching of the conidiophore and the globular shaped spores. Its low incidence on insects in nature, together with its low pathogenicity, indicates that probably it has little or no effect on the natural reduction of insect populations.

No. 11 Penicillium puberulum Bainier

Colonies on Czapek's solution agar growing more or less restrictedly, attaining a diameter of 3.0 to 3.5 cm. in 2 weeks at room temperature, with surface velvety to somewhat granular, raised and occasionally almost umbonate in central areas, with submarginal areas radiately wrinkled, azonate during the rapidly growing period but becoming more or less zonate in age and often showing a thin, spreading marginal area up to 1 cm. in width, closely but delicately zonate; growing margin white to light gray-green, 1mm. wide, quickly becoming darker, fruiting areas at first bluish-green but shading quickly to slate olive and finally to dark olive gray (Figure 13), at 2 to 3 weeks commonly showing an area of submerged growth up to 2mm. wide surrounding the colony and bearing scattered penicilli; reverse yellowish to tan to almost brownish black in colony center, with surrounding agar uncolored; exudate lacking or limited in amount, colorless; odor moldy to sourish, strong; conidiophores arising primarily from a tough basal mycelial felt, generally less than 200μ in length by 3.5 to 4.0μ wide, slightly sinuous with walls more or less roughened; penicilli asymmetric, consisting of a terminal verticil of metulae or of such a

verticil with branches and metulae arising from a lower node, often irregularly branched; branches usually 10 to 20μ by 2.8 to 3.5μ; metulae usually in groups of 2 to 4, ranging in dimensions from 9.0 to 15μ by 2.5 to 3.5μ; sterigmata usually in groups of 3 to 5 and measuring 7.0 to 9.0μ by 2.5 to 3.5μ, with form not distinctive, conidia globose to subglobose, with walls smooth or delicately roughened, mostly 3.0 to 3.5μ in diameter but variable in size up to 5.0 to 5.5μ.

P. puberulum was recovered from four different specimens of Ostrinia nubilalis. The species has not previously been reported from insects. It is distinguished from the other two species of Penicillium recovered from insects in this study by the asymmetrical branching of the conidiophores and the globose conidia.

Pathogenicity experiments conducted with P. puberulum gave mortality rates of from 0 to 25 percent, with an average of 15 percent for a series of five tests (Tables 2 and 3). The organism is no doubt a saprophyte or a low-grade pathogen.

Genus Rhizopus

The genus Rhizopus was established in 1820 by Ehrenberg. It is a common genus, cosmopolitan in nature, and found in many habitats. The description below was taken from Gilman (1957).

No. 12 Rhizopus stolonifer (Ehr. ex. Fr.) Vuillemin

Stolons creeping, recurving to the substrate in the form of arachnoid hyphae, which are strongly raised and distant from the substrate and implanted at each node by means of rhizoids. The internodes often attain a

length of 1 to 3 cm. and the hyphae are more or less branched. Sporangiophores rarely single, united in groups of three to five or more, 0.5 to 4 mm. in height by 24 to 42μ in diameter. Apophyses broad, cuneiform. Sporangia hemispheric 100 to 350μ (Figure 6 E and F). Columellae broad, hemispheric, depressed, 70μ in diameter by 90μ in height (250 by 320μ maximum). Spores unequal, irregular round or oval, angular, striate, 9 to 12μ long by 7.5 to 8μ in diameter, of a gray-blue. Zygospores round, or oval, 160 to 220μ in diameter. Exine brown-black, verrucose. Suspensors swollen, usually unequal. Azygospores present. No chlamydospores.

R. stolonifer was recovered from two larvae of the European corn borer, Ostrinia nubilalis. The two strains were tested for pathogenicity (Tables 2 and 3). One strain produced mortality rates of 37 to 87 percent in two tests conducted, while the other strain killed 22 to 25 percent of the larvae. The average mortality rate for the four tests was 42 percent. The organism is not common on corn insects in Iowa, but does seem to be weakly pathogenic for the European corn borer. The fungus has been previously recorded from the larva of a Sphingidae (Charles 1941).

Genus Scopulariopsis

This genus was established by Bainier in 1907 for a mold that is morphologically similar to Penicillium although probably not genetically related to that genus. Many species of this genus have been recorded, but no one has attempted to do a comparative study of the group as yet; therefore, the taxonomy of the genus is rather nebulous at this time. The following description was taken from Raper and Thom (1949).

No. 13 Scopulariopsis brevicaulis (Sacc.) Bainier

Colonies on Czapek's solution agar spreading rather broadly in most strains, more restricted in others, comparatively thin, plane, or irregularly but not deeply furrowed, at first grayish white, then avellaneous, or yellowish-brown even to light chocolate (Figure 7), with surface characterized by closely crowded, short conidiophores to produce powdery conidial areas overgrown by loosely trailing floccose hyphae and ropes of hyphae in most strains, deeper and less heavily sporing in others, with margin usually indeterminate and broadly spreading, azonate or broadly zonate from an uneven production of conidia. Conidiophores short, mostly 10 to 30μ (Figure 6 D), arising directly from the submerged hyphae, or irregularly borne as lateral and perpendicular branches from trailing aerial hyphae and ropes of hyphae. Conidial fructifications either simple and unbranched, sparingly branched, or consisting of verticillate and irregular branching systems bearing numerous divergent chains of conidia (Figure 6 A), often 150μ in length in old colonies. Sterigmatic cells often continuous with the conidiophores, variable, up to 20μ by about 3.0 to 4.0μ, sometimes tapering to slender conidium bearing tubes, in other cases essentially uniform in diameter throughout. Conidia somewhat pear-shaped (Figure 6 C), thick-walled, characteristically tuberculate but smooth when young and often appearing so in liquid mounts under oil immersion, commonly measuring 6.5 to 7.5μ by 7.5 to 9.0μ, avellaneous to light brown in mass, viable for several years, germinating by a single tube from the thin center of the broad base into a bulbous enlargement from which mycelial hyphae, about 2μ in diameter, arise.

Figure 6. Photomicrographs of entomogenous fungi

A. Scopulariopsis brevicaulis, conidiophore placement; approx. 600X

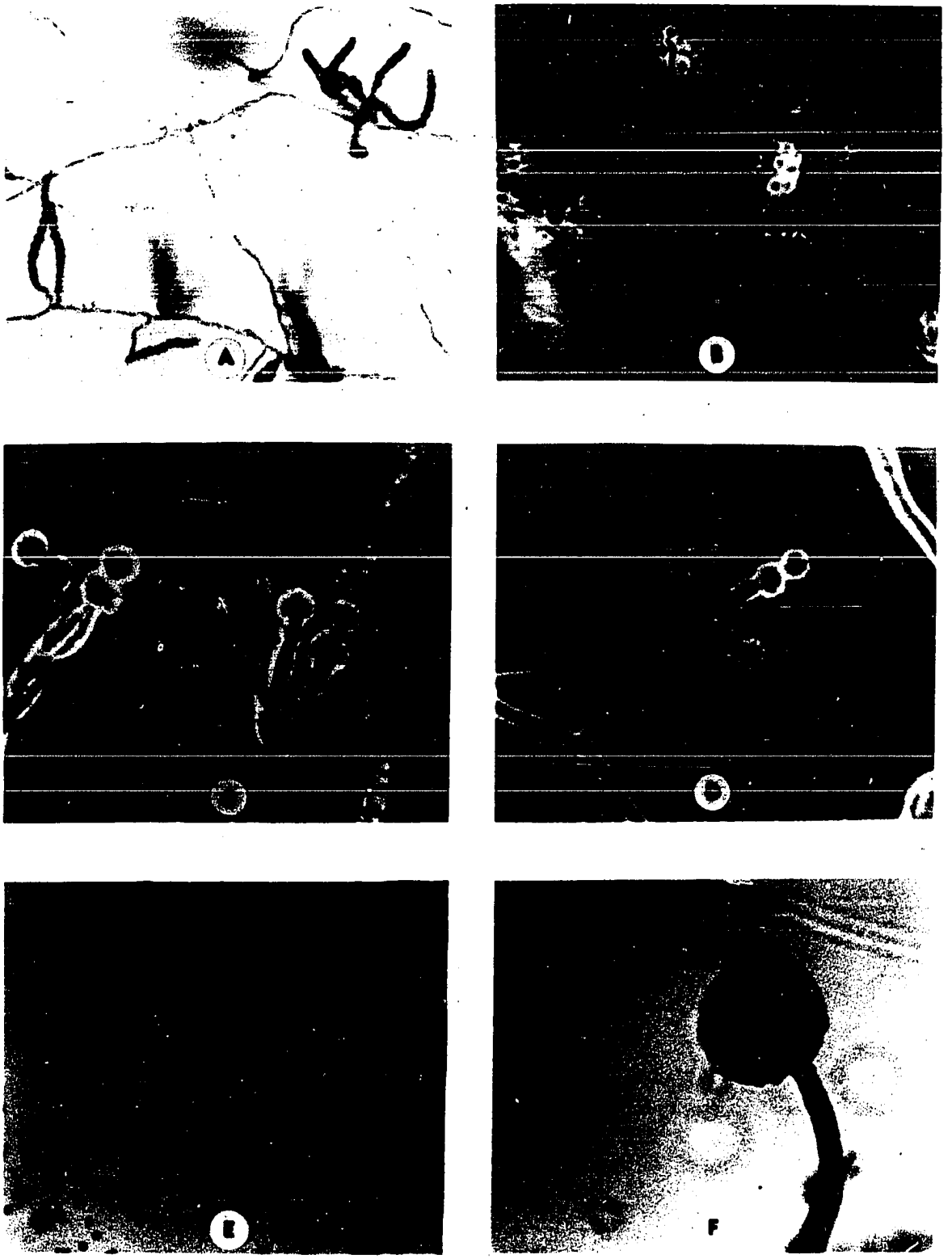
B. Scopulariopsis brevicaulis, conidiophores and conidia; approx. 1300X

C. Scopulariopsis brevicaulis, conidia with basal ring; approx. 3000X

D. Scopulariopsis brevicaulis, conidia; approx. 3000X

E. Rhizopus stolonifer, sporangiophore and spores; approx. 1300X

F. Rhizopus stolonifer, mature sporangia; approx. 1300X



This genus has frequently been reported as parasitic on man and other vertebrate animals, but until now has not been reported on insects. The genus causes the disease "American Blastomycosis" in humans (Raper and Thom 1949).

This organism was recovered from 14 pupae of the corn earworm, Heliothis zea, all taken in one spot from soil in one of the rearing cages at Ankeny, Iowa. The spores form a light brown powdery covering over almost the entire insect cadaver (Figure 8).

Two pathogenicity tests were run with this organism (Tables 2 and 3), and mortality rates of 33 and 43 percent were obtained, using larvae of the European corn borer as test animals. This organism is weakly pathogenic for Ostrinia nubilalis but may be more effective against the host from which it was recovered. Larvae of Heliothis zea were not available for testing purposes.

Figure 7. Fungi growing on *Ostrinia nubilalis*

(Left to right)

Scopulariopsis brevicaulis

Beauveria bassiana

Paecilomyces varioti

Fusarium neoceras

Figure 8. Fungi growing on *Ostrinia nubilalis*

(Left to right)

Metarrhizium anisopliae

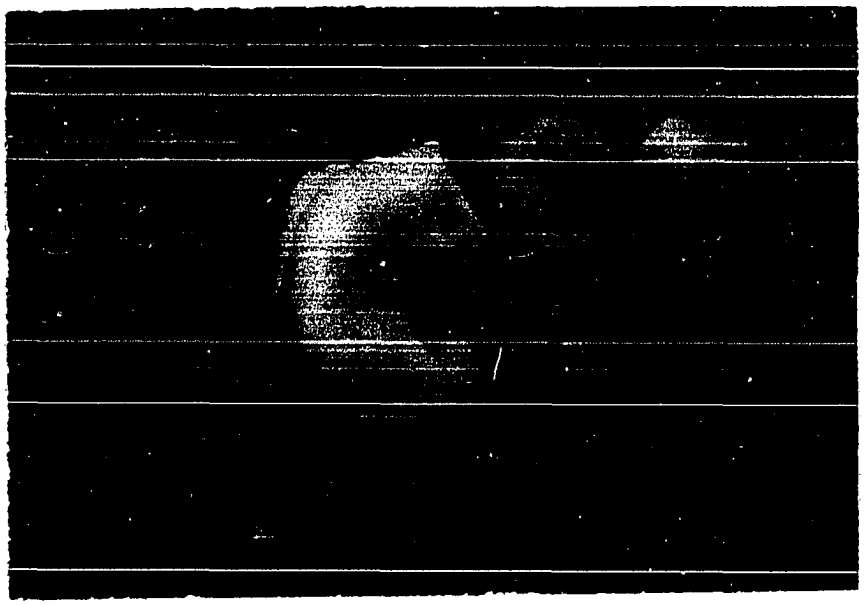
Scopulariopsis brevicaulis

Paecilomyces varioti

Beauveria bassiana

Aspergillus ustus

Rhizopus stolonifer



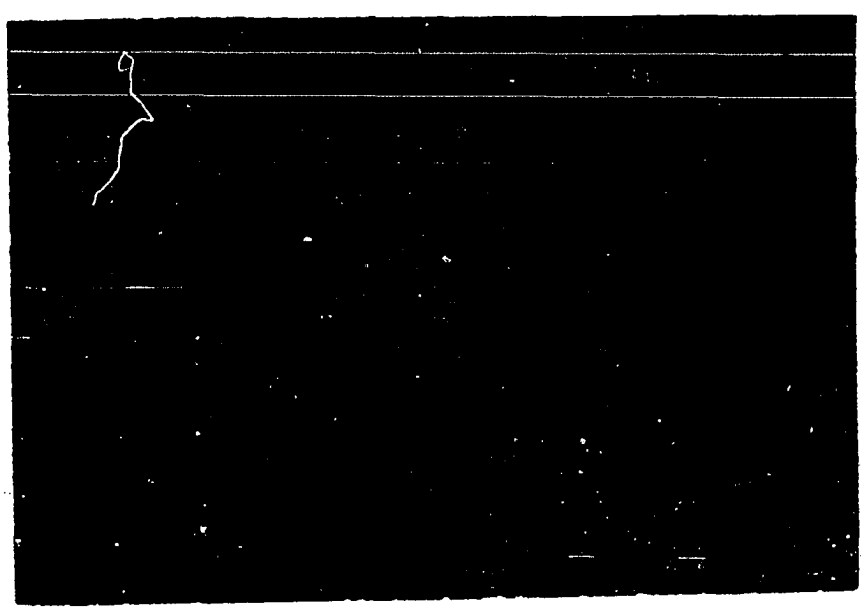
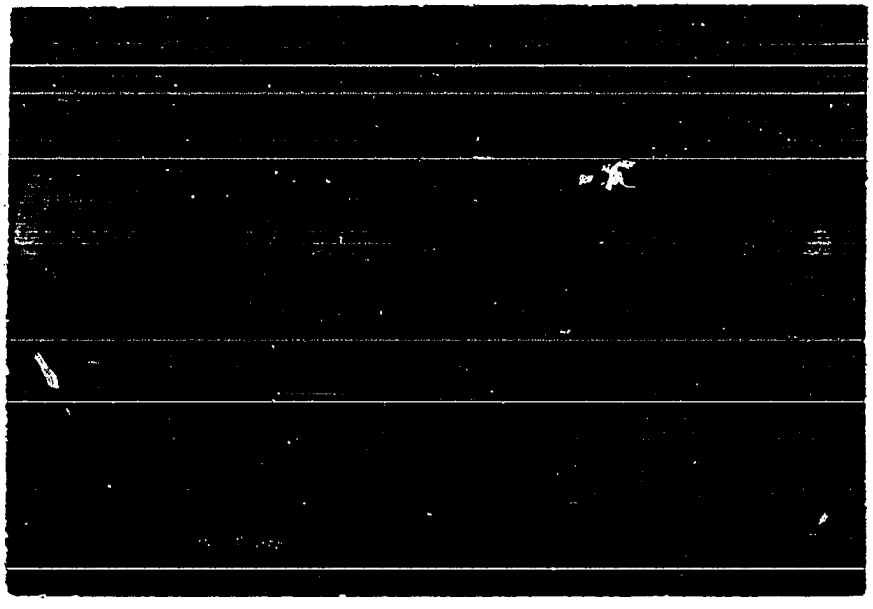


Figure 9. Fungi growing on Ostrinia nubilalis

Top: Metarrhizium anisopliae

Bottom: Aspergillus parasiticus

Figure 10. Aspergillus niger growing on Ostrinia nubilalis



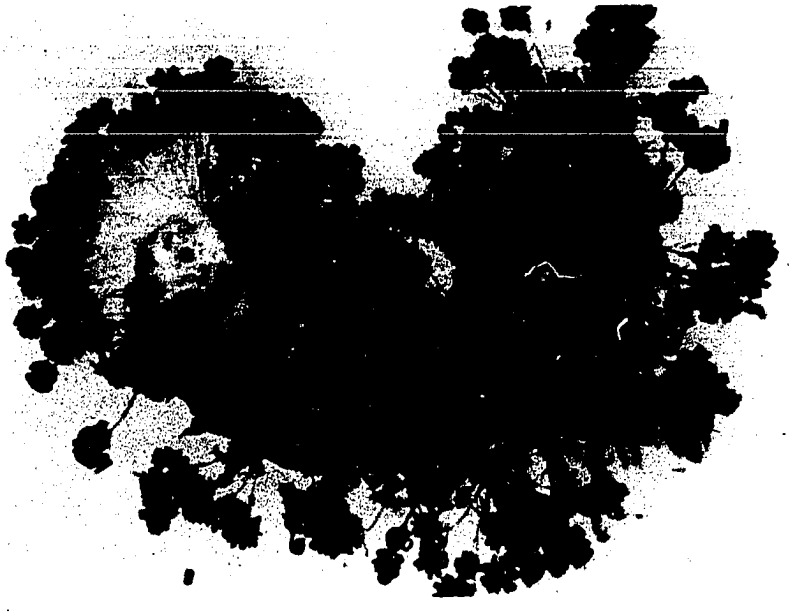


Figure 11. Beauveria bassiana growing on Diabrotica longicornis

Figure 12. Beauveria bassiana growing on Glischrochilus quadrisignatus

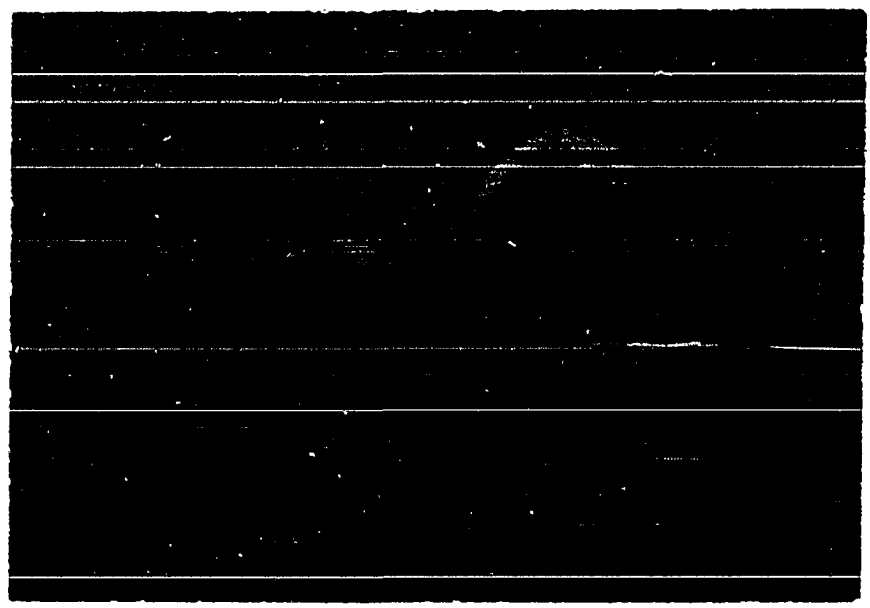
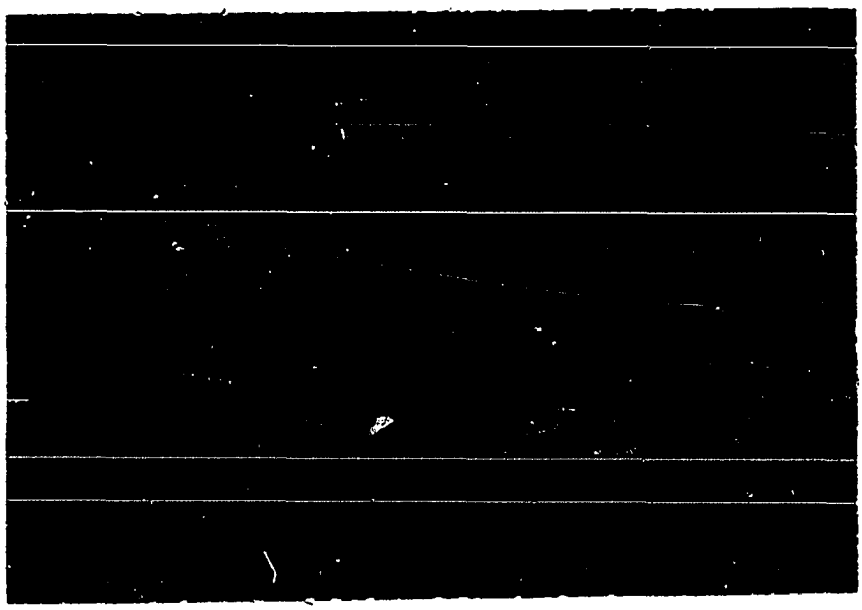


Figure 13. *Penicillium* cultures on artificial media

Upper left: *P. cyclopium*

Lower left: *P. puberulum*

Right: *P. decumbens*



CONCLUSIONS

The results of this study on fungi isolated from corn insects indicate that many more species of fungi are entomogenous than are reported in the literature on the subject.

Many fungi normally considered to be soil fungi grew readily on dead insects. Some of them were parasitic and pathogenic. Others were strictly saprophytic, using the dead insect as nutritive material.

It was concluded from this study that some of these organisms (Beauveria bassiana, Metarrhizium anisopliae, Aspergillus parasiticus, and Fusarium neoceras) were sufficiently abundant and pathogenic to be of value in the natural reduction of wild insect populations.

It was apparent that certain species could become important as pathogens in insect rearing programs. The mold inhibitor in the artificial diet of laboratory-reared European corn borer larvae did not deter Aspergillus parasiticus from infesting and killing the larvae.

The only fungi recovered during this study which produced sufficiently high mortality rates to be considered of value as microbial insecticides were B. bassiana and M. anisopliae.

SUMMARY

Dead insects, collected in various parts of the state of Iowa, were examined in the laboratory for symptoms or signs of mycosis. Those infected with fungi were routinely processed to isolate the fungi concerned.

The isolated organisms were grown on culture slides, or were mounted for microscopic examination, to identify the species of fungus involved.

Pathogenicity experiments were conducted with each of the fungus strains that was isolated. Larvae of the European corn borer, Ostrinia nubilalis, were used as test insects in all of the experiments. Two different treatment methods were employed in these tests. The first type involved a topical application of a water suspension of spores to the epidermis of the test larvae using a microinjector. The second method caused the larvae to crawl through a pure culture of the fungus to be tested.

Beauveria bassiana, and Metarrhizium anisopliae were found to be the most pathogenic organisms recovered in this investigation. Aspergillus parasiticus, and Fusarium neoceras were less pathogenic but much more common among the wild insect populations. Other fungi recovered from dead corn insects during the course of this study were found to be less virulent and not abundant in nature. These were: Aspergillus niger, Aspergillus ustus, Mycoderma clayi, Paecilomyces varioti, Penicillium cyclopium, Penicillium decumbens, Penicillium puberulum, Rhizopus stolonifer, and Scopulariopsis brevicaulis. Aspergillus ustus, Fusarium neoceras, Paecilomyces varioti, Penicillium decumbens, Penicillium puberulum, and Scopulariopsis brevicaulis have not previously been reported from insects.

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ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. Earle Raun, professor in charge of major work, for his encouragement, guidance, and assistance during the course of this investigation; and to Dr. Lois Tiffany for her patience and assistance in the identification of the fungi discussed in this study.

This work was carried out under Project No. 1438, which is supported in part by Contract No. 12-14-100-2603(33) with the Entomology Research Division, Agricultural Research Service, U. S. Dept. of Agriculture.

The author is indebted to Dr. T. A. Brindley for making available the research facilities of the European Corn Borer Research Laboratory at Ankeny, Iowa, and for supplying the thousands of larvae needed for the tests which were conducted in the course of this investigation.

Thanks are also due my many friends and colleagues here at the university who freely offered advice, assistance, and equipment needed for the successful completion of this work.

Lastly, I wish to acknowledge the assistance and the encouragement of my understanding wife, JaNa, without whose help the completion of these years of study would not have been possible.

APPENDIX

A List of Media Used in this Study

1. Corn meal agar

Ingredients:

Corn meal	40 gms.
Water	1000 ml.
Agar	20 gms.
Glucose	10 gms.

Simmer corn meal and water for 1 hour. Filter, and bring volume back up to 1000 ml. and add agar and glucose; heat to melt agar and glucose and then autoclave.

2. Czapek's agar

Ingredients:

Sucrose	30 gms.
Sodium nitrate	3 gms.
Dipotassium phosphate	1 gm.
Magnesium sulfate	0.5 gm.
Potassium chloride	0.5 gm.
Ferrous sulfate	0.01 gm.
Water	1000 ml.
Agar	15 gms.

A commercial preparation can be obtained, or the medium may be mixed with the above ingredients. Mix all of the ingredients and heat until melted and then autoclave.

3. Brain-heart Infusion Agar

Ingredients:

Calf brain	200 mg.
Beef heart, infusion from	250 gms.
Proteose peptone, Difco	10 gms.
Dextrose	2 gms.
NaCl	5 gms.
Disodium phosphate	2.5 gms.
Agar	15 gms.
Water	1000 ml.

A commercial preparation can be obtained, or the medium may be mixed with the above ingredients.

4. Littman Oxgall Agar

Ingredients:

Bactopeptone	10 gms.
Dextrose	10 gms.
Bacto-oxgall	15 gms.
Agar	20 gms.
Crystal violet	0.01 gms.
Water	1000 ml.

A commercial preparation can be obtained, or the medium may be mixed with the above ingredients.

5. Sabouraud's Agar

Ingredients:

Glucose	40 gms.
Neopeptone	10 gms.
Agar	15 gms.
Water	1100 ml.

Commercial preparations are available, or the medium may be mixed using the above ingredients.

6. Nutrient Agar

Ingredients:

Bacto beef extract	3 gms.
Bacto peptone	5 gms.
Agar	15 gms.
Water	1000 ml.

Can be obtained commercially or mixed.

7. Potato Dextrose Agar

Ingredients:

Potato infusion	200 gms.
Dextrose	20 gms.
Agar	15 gms.
Water	1000 ml.

8. Blood Agar

Ingredients:

A commercial preparation base agar is obtained (DIFCO # B44) and to this is added:

Fresh blood	60 cc
Water	1000 ml

9. Thompson's Agar

Ingredients:

Potato	100 gms.
Bacto peptone	10 gms.
Active Dry Yeast	10 gms.
Fresh egg yolk	10 gms.
Agar	15 gms.
Water	1000 ml.

Table 4. Information on exposures of the photomicrographs

Figure No.	Exposure time	Objective lens	Film type	Mount type[a]
	Seconds	x		
3 A	1/10	10	Kodachrome II	C. S.
B	1/5	20	Ektachrome Type F	C. S.
C	1/2	43	do.	C. S.
D	1/2	97	do.	C. S.
E	1/2	43	do.	C. S.
F	1/2	10	do.	C. S.
4 A	1/2	43	Ektachrome Type F	C. S.
B	1/5	43	do.	C. S.
C	1	97	do.	L. P.
D	1/2	43	do.	C. S.
E	1	43	Kodachrome	O. P.
F	1	97	Ektachrome Type F	L. P.
5 A	1	97	Kodachrome II	L. P.
B	1/2	20	Panatomic X	C. S.
C	2	97	Kodachrome	L. P. C. B.
D	1	43	Kodachrome II	L. P.
E	1	97	do.	L. P.
F	1	43	do.	C. S.
6 A	1/2	20	Ektachrome Type F	C. S.
B	1/2	43	Ektachrome Type F	C. S.
C	1/2	97	do.	C. S.
D	1	97	do.	C. S.
E	1/5	43	do.	L. P.
F	1	43	do.	C. S.

[a]C. S. = Culture Slide.
L. P. = Lacto-Phenol mounted specimen.
L. P. C. B. = Lacto-Phenol mounted specimen, stained with Cotton Blue.
O. P. = On plate, culture was photographed on an agar plate.

Table 5. Information on the close-up photographs

Figure No.	F-Stop	Exposure time Seconds	Film type
7	F-22	1	Kodachrome II
8	F-11	1	do.
9	F-16	1	High Speed Ektachrome
10	F-8	1	Kodachrome II
11	F-11	1	do.
12[a]			
13	F-11	1/2	High Speed Ektachrome

[a]Taken by Robert D. Jackson, U. S. Dept. of Agriculture